

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE April 2004	3. REPORT TYPE AND DATES COVERED Annual (1 Apr 2003 - 31 Mar 2004)	
4. TITLE AND SUBTITLE Detection of Serum Lysophosphatidic Acids Using Affinity Binding and Surface Enhanced Laser Desorption/Ionization (SELDI) Time of Flight Mass Spectrometry			5. FUNDING NUMBERS DAMD17-03-1-0222	
6. AUTHOR(S) Gordon B. Mills, M.D.			8. PERFORMING ORGANIZATION REPORT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The University of Texas M.D. Anderson Cancer Center Houston, TX 77030 E-Mail: gmills@mdanderson.org				
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) <p>We proposed to apply two novel technologies to the development of an approach suitable for screening for ovarian cancer in high and low risk women. The first of these is a novel approach to the development of antibodies which will recognize specific phospholipids and lysophospholipids present in ovarian cancer patients and the second of these is SELDI tof mass spectroscopy. These two technologies will be merged with powerful computing tools to develop approaches capable of detecting ovarian cancer at an early, curable stage. This approach will further benefit from the expertise of the Mills laboratory (LPA screening, SELDI tof) with that of the Prestwich laboratory (lipid synthesis and antibody development).</p> <p>Progress</p> <p>We have demonstrated that SELDI mass spectroscopy has the ability to detect model lysophospholipids present in plasma and serum. The sensitivity of the assay using standard capture chips is low and would require relatively large volumes of sera to detect the different lysophospholipid isoforms. We have obtained a pan sphingosine 1 phosphate antibody as a capture reagent. This antibody is able to bind all isoforms of S1P and when combined with SELDI should allow detection of this lysophospholipid. We are currently evaluating different CHIP forms for SELDI and developing LPA antibodies as well as obtaining LPA binding proteins to increase the ability to capture lysophospholipids.</p>				
14. SUBJECT TERMS Ovarian cancer, LPA, SELDI, early diagnosis				15. NUMBER OF PAGES 20
				16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
ANSI Std. Z39-18

20041021 016

AD _____

Award Number: DAMD17-03-1-0222

TITLE: Detection of Serum Lysophosphatidic Acids using Affinity
Binding and Surface Enhanced Laser Desorption/Ionization
(SELDI) Time of Flight Mass Spectrometry

PRINCIPAL INVESTIGATOR: Gordon B. Mills, M.D.

CONTRACTING ORGANIZATION: The University of Texas
M.D. Anderson Cancer Center
Houston, TX 77030

REPORT DATE: April 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

Table of Contents

Cover.....	
SF 298.....	1
Table of Contents.....	2
Introduction.....	3
Body.....	5
Key Research Accomplishments.....	9
Reportable Outcomes.....	9
Conclusions.....	9
References.....	9
Appendices.....	9

INTRODUCTION

Background and Preliminary Data

Ovarian cancer remains the 5th most frequent cause of death from cancer in women. Indeed, the current cure rate for ovarian cancer is under 40%, not substantially different from that in the 1950s. This abysmal prognosis occurs, in most part, due to ovarian cancer being diagnosed at a late stage where current therapies are ineffective. Unfortunately more than 75% of patients are diagnosed when the disease has spread beyond the pelvis. Thus a method to detect ovarian cancer at an early curable stage has the potential to provide an immediate and major impact on this devastating disease. This is particularly important for individuals at high risk either because of a strong family history or proven abnormalities in BRCA1 or BRCA2.

The identification of secreted molecules, which contribute to the pathophysiology of ovarian cancer, provides a major opportunity to identify markers that could contribute to early diagnosis. We have demonstrated that the most potent growth factor activity in ascites of ovarian cancer patients consists of multiple forms of lysophosphatidic acid (LPA). LPA increases proliferation, prevents apoptosis and anoikis, increases invasiveness, decreases sensitivity to cisplatin (the most effective drug in ovarian cancer), and increases production and activity of multiple growth factors, proteases and mediators of angiogenesis. Thus LPA contributes to the pathophysiology of ovarian cancer. We and others have subsequently demonstrated that multiple additional bioactive lysophospholipids, including lysophosphatidylcholine (LPC), sphingosylphosphorylcholine (SPC), sphingosine 1 phosphate (S1P) and lysophosphatidylserine (LPS) exhibit pleiomorphic effects on ovarian cancer cells.

Ascites from ovarian cancer patients contains high levels of lysophospholipids including multiple forms of LPA, lysophosphatidylinositol (LPI), LPC, SPC and S1P. Reports from our and other laboratories indicate that plasma and sera from ovarian cancer patients contain aberrant levels of these lysophospholipids. This suggests that an efficient method to determine levels of lysophospholipids in serum or plasma could provide an effective method to screen for ovarian cancer.

Over 500 species of lysophospholipids are present in plasma and serum. Currently, lysophospholipids are quantified by lipid extraction of a serum sample followed by Mass spectrometry (MS). While MS analysis is highly accurate, the methodology has limited throughput for large-scale screening of patient samples. Further, the need to use organic solvents to prepare specimens for MS limits the quantitation and the applicability of the method. Surface-enhanced laser desorption and ionization time of flight (SELDI tof) mass spectroscopy analyses of "markers" diagnostic of ovarian cancer have identified a number of candidates with mass/charge ratios of under 1000, suggestive that these could be lysophospholipids rather than peptides. Identification of these markers required the global unbiased analysis of many different molecules in serum combined with powerful algorithms designed to identify patterns indicative of the presence of cancer.

We propose to apply two novel technologies to the development of a high through-put technology suitable for screening for ovarian cancer in high and low risk women. The first of these is a novel approach to the development of antibodies to

specific phospholipids and lysophospholipids and the second of these is SELDI tof mass spectroscopy. These two technologies will be merged with powerful bioinformatics tools to develop heuristic algorithms capable of detecting ovarian cancer at an early, curable stage. This approach will benefit from the expertise of the Mills laboratory (LPA screening, SELDI tof) with that of the Prestwich laboratory (lipid synthesis and antibody development).

To develop efficient methods for analysis of lysophospholipids in ovarian cancer patients, we will:

- 1. Assess the efficacy of novel LPA/PA lipid antibodies developed by our group in capture and analysis of LPA/PA directly in serum and plasma using SELDI-tof**
- 2. Determine whether non-specific matrices (hydrophobic C16, anionic SAX2) can be used to directly determine phospholipid and lysophospholipid levels using SELDI-tof**
- 3. Develop additional anti lysophospholipid antibodies and determine their utility in analysis in Seldi-tof**

Significance: Over 75% of ovarian cancer patients are diagnosed when the disease has spread beyond the pelvis. At this stage of disease, the cure rate is under 15%. This is in contrast to the cure rate for early stage ovarian cancer, which can approach 90%. Thus any approach that can allow diagnosis of ovarian cancer at an earlier curable stage has the potential to have a marked impact on this devastating disease.

BODY

Statement of Work

Task #1 Assess the efficacy of novel LPA/PA lipid antibodies developed by our group in capture and analysis of LPA/PA directly in serum and plasma using SELDI-tof (months 1-24)

This specific aim was dependent on the production of anti-lipid antibodies and identification of LPA and PA binding proteins for selective capture of these ligands from serum and ascites. Recently, we have experienced difficulty in achieving selective lipid binding by anti-lipid antibodies, scale-up production issues, and problems in obtaining homogenous, healthy anti-lipid producing clones. These are problems of significant general concern for developing a robust analytical method.

Thus, a modification of the initial aim would involve the broadening to include discovery of additional lysophospholipid and lipid binding proteins that could be readily produced as recombinant proteins and engineered to have particular useful lipid binding characteristics. To this end, we have employed our tethered LPA and PA reagents, used earlier for anti-lipid antibody elicitation, to make affinity resins for identification of novel LPA binding proteins from fibroblasts and cancer cells. We plan to do the same modification for LPC, PC, LPI, and PI lipids. To identify new lysophospholipid binding proteins, a MS proteomics collaboration was established with Drs. J. Gettemans and J. Vanderkerckhove at the University of Ghent (Belgium). This collaboration has the benefit of discovering novel proteins in the LPA signaling pathway, which are likely to be important as diagnostic markers in their own right, as well as being important reagents for the proposed SELDI-MS capture method. The collaboration will be broadened with other lysophospholipids as the reagents become available. The engineering of new specificities by mutagenesis will be incorporated later in this program, following identification of scaffold lipid-binding proteins and determination of their 3D structures.

Although, there has been difficulty in development of sufficient amounts of LPA/PA antibodies for this aim, we have obtained a high affinity pan S1P antibody from Dr. Roger Sabadinni at UCSD. This antibody binds all forms of S1P and demonstrates efficacy in determining S1P levels using ELIZA. However, the ELIZA approach only determines the total isoforms of S1P present and does not allow identification of the S1P isoforms present. As described for the LPA antibodies, we are coupling the S1P antibodies to the Seldi-tof matrices. We are also working with Dr. Sabadinni to produce anti-LPA antibodies using the approaches developed for making anti-S1P antibodies.

Either the LPA binding protein or LPA antibody approaches are expected to be successful. We will proceed with the tasks listed herein with the S1P antibodies.

1.1 Couple antibodies to Seldi-tof matrices (Months 1-4)

1.2 Validate Seldi tof analysis with model lysophospholipids/phospholipids (Months 4-8)

1.3 Validate Seldi tof analysis with sera and plasma with known lipid composition (Months 8-14)

1.4 Validate quantification of Seldi tof analysis with stable isotope labeled lysophospholipid/lipid spiked into plasma and serum (Months 14-20)

1.5 Validate Seldi tof analysis with teaching and training set of serum and plasma samples from ovarian cancer patients (Months 20-24)

Each of the subsequent steps in this Task will be dependent on the successful completion of the preceding Task

Task #2 Determine whether non-specific matrices (hydrophobic C16, anionic SAX2) can be used to directly determine phospholipid and lysophospholipid levels using SELDI-tof

We have made significant process in this task. We have applied model lysophospholipids to matrices and demonstrated an ability to detect the model lysophospholipids in sera. We can readily detect 0.2nmol of LPA and other lysophospholipid isoforms in a single spot using the SELDI matrix. Total LPA levels in plasma, sera and ascites are between 100nM and 80µM. These are readily detectable requiring a maximum of 2ml of plasma, and very low amounts of sera and ascites. However, in order to detect isoforms of LPA that may be present at much lower concentrations, we would need however as much as 7ml of plasma loaded on to the current matrix. Thus we are now assessing different matrices and washing conditions. Nevertheless, 7 ml of plasma could be obtained from patients for analysis. This will require a pre purification step before loading onto the Seldi matrix. This is currently in progress.

PA and LPC are present at much higher levels than LPA, allowing ready detection. LPI and LPE are present at similar levels to LPA suggesting that the approach is on target and will be able to detect the multiple different lipids present in plasma, sera and ascites.

2.1 Identify and obtain matrices for analysis (months 1-4)

2.2 Determine conditions for binding of lysophospholipids/phospholipids (months 5-8)

2.3 Determine conditions for washing of lysophospholipids/phospholipids (months 9-12)

2.4 Select affinity matrix for further analysis (month 12)

2.5 Validate Seldi tof analysis with model lysophospholipids/phospholipids (Months 13-16)

2.6 Validate Seldi tof analysis with sera and plasma with known lipid composition (Months 16-20)

2.7 Validate quantification of Seldi tof analysis with stable isotope labeled lysophospholipids/phospholipids spiked into plasma and serum (Months 21-24)

2.8 Validate Seldi tof analysis with teaching and training set of serum and plasma samples from ovarian cancer patients (Months 24-28)

Task #3 Develop additional anti-lysophospholipid/lipid antibodies and determine their utility in analysis in Seldi-tof (1-36)

As indicated in the description for Task#1. We have already obtained S1P antibodies. Further as indicated in Task#1, we are switching the emphasis of Task#3 to obtaining high affinity lipid binding proteins using the tethered lysophospholipid moieties developed for immunization of mice. To this, end, we have employed our tethered LPA and PA reagents, used earlier for antilipid antibody elicitation, to make affinity resins for identification of novel LPA binding proteins from fibroblasts and cancer cells. We plan to do the same modification for LPC, PC, LPI, and PI lipids. To identify new lysophospholipid binding proteins, a MS proteomics collaboration was established with Drs. J. Gettemans and J. Vanderkerckhove at the University of Ghent (Belgium). This collaboration has the benefit of discovering novel proteins in the LPA signaling pathway, which are likely to be important as diagnostic markers in their own right, as well as being important reagents for the proposed SELDI-MS capture method. The collaboration will be broadened with other lysophospholipids as the reagents become available. The engineering of new specificities by mutagenesis will be incorporated later in this program, following identification of scaffold lipid-binding proteins and determination of their 3D structures.

3.1 Develop immunogens for lysophosphatidyl choline/phosphatidylcholine (LPC/PC) (months 1-4)

3.2 Immunize with LPC/PC immunogen (months 5-8)

3.2 Select antibodies to LPC/PC for coupling to matrices for Seldi tof (months 9-12)

3.3 Develop immunogens for lysophosphatidylinositol/phosphatidylinositol (LPI/PI) (months 5-8)

3.4 Immunize with LPI/PI immunogen (months 9-12)

3.5 Select antibodies for LPI/PI for coupling to matrices for Seldi tof (months 13-16)

3.6 Develop immunogens for sphingosine 1 phosphate/sphingosylphosphorylcholine (S1P/SPC) (months 13-16)

3.7 Immunize with S1P/SPC immunogen (months 17-20)

3.8 Select antibodies to S1P/SPC for coupling to matrices for Seldi tof (months 21-24)

3.9 Analyze antibodies with Seldi-tof This process will follow the description in task 1.
The expertise gained in task 1 will facilitate the rapid completion of this step
(Months 24-36)

KEY RESEARCH ACCOMPLISHMENTS

1. Demonstrated that model lysophospholipids can be detected at concentrations present in patients by SELDI tof
2. Obtained sufficient quantities of a high affinity S1P antibody for assessment by SELDI tof
3. Initiated identification of high affinity lysophospholipid binding proteins.

REPORTABLE OUTCOMES

None

CONCLUSIONS

Overall, there has been significant progress in achieving the aims of the proposal. We have obtained a high affinity S1P antibody and are linking it to appropriate matrices. We have demonstrated that Seldi ToF has the ability to detect the amounts of lysophospholipids present in plasma, sera and ascites. The proposed improvements in the approach should increase the sensitivity of the assay to allow the detection of different isoforms in plasma. Increased sensitivity is not required for sera or ascites.

REFERENCES

- Mills G.B., and Moolenaar, WH. 2003 Emerging role of lysophosphatidic acid in cancer. *Nature Cancer Reviews* 3:582-591
- Goto, M., Tanyi, J., Lahad, J., Liu, S., Yu, S., Lapushin, R., Hasegawa, Y., Lu, Y., Trost, R., Bevers, T., Jonasch, E., Aldape, K., Liu, J., James, R.A., Ferguson, C.G., Xu, Y., Prestwich, G.D., and Mills G.B., 2004 Lysophosphatidic acid production and action: Validated targets in cancer. *J. Cellular Biochemistry* In Press.

APPENDICES

- Mills G.B., and Moolenaar, WH. 2003 Emerging role of lysophosphatidic acid in cancer. *Nature Cancer Reviews* 3:582-591

THE EMERGING ROLE OF LYSOPHOSPHATIDIC ACID IN CANCER

Gordon B. Mills* and Wouter H. Moolenaar†

The bioactive phospholipid lysophosphatidic acid (LPA) stimulates cell proliferation, migration and survival by acting on its cognate G-protein-coupled receptors. Aberrant LPA production, receptor expression and signalling probably contribute to cancer initiation, progression and metastasis.

The recent identification of ecto-enzymes that mediate the production and degradation of LPA, as well as the development of receptor-selective analogues, indicate mechanisms by which LPA production or action could be modulated for cancer therapy.

PHOSPHOLIPID

A small molecule with one or two fatty acyl chains, a glycerol backbone, and a free or derivatized phosphate. Lysophospholipids only have a single fatty acyl chain (see figure 1).

SERUM

Fluid produced during blood coagulation. Many growth factors and mediators are released by platelets during clotting.

ECTO-ENZYME

An enzyme that is located on the outside of the cell. Primarily involved in metabolism of molecules in the interstitial space or bloodstream.

*MD Anderson Cancer Center, Houston 77030, Texas, USA.

†The Netherlands Cancer Institute, Amsterdam 1066CX, The Netherlands. Correspondence to G. B. M. e-mail: gmills@mdanderson.org doi:10.1038/nrc1143

Lysophosphatidic acid (LPA) is one of the simplest natural PHOSPHOLIPIDS and, arguably, is also one of the most interesting. It consists of a single fatty acyl chain, a glycerol backbone and a free phosphate group, and unlike most other phospholipids, it is also water soluble (FIG. 1). Despite its simplicity, many structurally diverse forms of LPA exist, so it has the potential to contain a remarkable amount of informational content.

Although originally known for its rather unglamorous role as an intermediate in intracellular lipid metabolism, LPA is now recognized as an extracellular lipid mediator that evokes growth-factor-like responses in almost every cell type, both normal and transformed. The first indication that LPA was an important bioactive lipid came several decades ago, when it was found to induce smooth-muscle contraction, platelet aggregation and alterations in blood pressure, but the significance and physiological implications of those early findings remained obscure until the late 1980s and early 1990s. At this time, LPA was shown to have growth-factor-like activities, to signal through specific cell-surface receptors in a G-protein-dependent manner, and to be a major active constituent of SERUM. A new era of LPA research therefore began, and this has led to the unravelling and biochemical characterization of LPA's multiple signalling pathways and the discovery of new biological actions.

As an inducer of cell proliferation, migration and survival, LPA's actions are concordant with many of the 'hallmarks of cancer'¹, indicating a role for LPA in the initiation or progression of malignant disease. Indeed, LPA levels are significantly increased in malignant effusions, and its receptors are aberrantly expressed in several human cancers. The most noteworthy recent development underscoring the importance of LPA in cancer, however, is the discovery that a previously enigmatic ECTO-ENZYME that is involved in tumour invasion, neovascularization and metastasis — autotaxin (ATX) — acts by producing LPA in the cellular microenvironment, which indicates that LPA is a key contributor to the metastatic cascade.

Biological actions of LPA

The list of cellular responses to LPA is remarkably diverse (TABLE 1). Several of LPA's actions are rapid — for example, it affects morphological changes, motility, chemotaxis, invasion, gap-junction closure and tight-junction opening — and occur independently of new protein synthesis. Others are long-term and secondary to gene transcription, such as the stimulation of cell-cycle progression, increased cell viability, wound healing, the production of endothelin and pro-angiogenic factors (vascular endothelial growth factor (VEGF), interleukin (IL)-6, IL-8 and GRO1) — which can act as paracrine growth factors for malignant cells and can

Summary

- Lysophosphatidic acid (LPA) is a serum phospholipid with growth-factor-like activities for many cell types. It acts through specific G-protein-coupled receptors on the cell surface.
- LPA stimulates cell proliferation, migration and survival. In addition, LPA induces cellular shape changes, increases endothelial permeability and inhibits gap-junctional communication between adjacent cells. LPA promotes wound healing *in vivo* and suppresses intestinal damage following irradiation.
- LPA receptors couple to multiple signalling pathways that are now being clarified. These pathways include those initiated by the small GTPases RAS, RHO and RAC, with RAS controlling cell-cycle progression and RHO/RAC signalling having a dominant role in (tumour) cell migration and invasion.
- Significant levels (>1 μM) of bioactive LPA are detected in various body fluids, including serum (but not plasma), saliva, follicular fluid and malignant effusions. The mechanisms by which bioactive LPA is produced were unknown until recently.
- Recent evidence shows that LPA is produced extracellularly from lysophosphatidylcholine by 'autotaxin' (ATX/lysoPLD). ATX/lysoPLD is a ubiquitous *exo*-phospholipidase that was originally identified as an autocrine motility factor for melanoma cells and is implicated in tumour progression. Through local production of bioactive LPA, ATX/lysoPLD might support an invasive microenvironment for tumour cells and therefore contribute to the metastatic cascade.
- Both LPA receptors and ATX/lysoPLD are aberrantly expressed in several cancers.
- The use of inhibitory drugs directed against LPA receptors and/or ATX/lysoPLD could be effective in suppressing tumour metastasis.

METALLOPROTEINASE

A class of metal-ion-requiring extracellular proteases.

G-PROTEIN-COUPLED RECEPTOR

A cell-surface receptor for small molecules, peptides and lipids that spans the plasma membrane seven times and signals via heterotrimeric G proteins.

NECROSIS

A form of cell death that is distinguished by autolysis.

ISCHAEMIA-REPERFUSION INJURY

Cellular injury that occurs when hypoxic tissue is reoxygenated. A significant problem in stroke, heart attacks and kidney injury.

MILDLY OXIDIZED LDL

Low-density lipoprotein (LDL) is present in plasma. It is a large spherical particle that is made up of cholesterol, cholesteryl esters, phospholipids and a single protein that organizes the particle. Under oxidative stress, LDLs become modified, resulting in alterations in lipid composition.

ATHEROSCLEROSIS

Narrowing of the blood vessels due to deposition of 'plaque' following injury. It is a frequent cause of cardiovascular disease, including heart attacks and stroke.

also alter the *in vivo* environment by increasing neovascularization — and the production or activation of proteases such as urokinase plasminogen activator (uPA), METALLOPROTEINASES (such as MMP-2) and the metalloprotease-disintegrin tumour necrosis factor- α converting enzyme (TACE)²⁻²⁹.

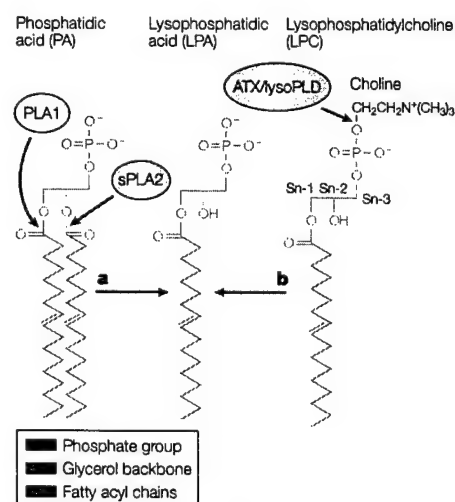


Figure 1 | LPA metabolic pathways. LPA can be produced by at least two distinct enzymatic mechanisms: **a** | hydrolysis of phosphatidic acid (PA) by soluble phospholipase A2 (sPLA2), which cleaves the fatty acyl chain at the sn-2 position, or hydrolysis by phospholipase A1 (PLA1), which cleaves the fatty acyl chain at the sn-1 position of the glycerol backbone; **b** | or hydrolysis of lysophosphatidylcholine (LPC) by ATX/lysoPLD, which liberates the hydrophilic headgroup (choline). LPC is produced by the action of PLA1 or PLA2 on membrane phosphatidylcholine.

In addition to transcriptional activation of growth factors, one mechanism by which LPA could indirectly regulate cellular function is through a G-PROTEIN-COUPLED RECEPTOR (GPCR)-regulated transmembrane metalloproteinase cleaving the precursor heparin-binding epidermal growth factor (HB-EGF) or amphiregulin protein at the cell surface, thereby allowing 'transactivation' of the EGF receptor in a classic autocrine manner^{24,30,31}. Human EGF-receptor (HER) family members are frequently overexpressed in cancer. Indeed, LPA induces tyrosine phosphorylation of many intracellular proteins, including members of the HER family^{5,16,30-33}, which is compatible with the idea of LPA inducing a number of cellular responses through the release and action of ligands for the HER family and other tyrosine-kinase-linked receptors.

Although in most circumstances LPA seems to increase cell viability, LPA can induce NECROSIS and apoptosis in hippocampal neurons and neuronal PC12 cells³¹⁻³⁵. Furthermore, renal ISCHAEMIA-REPERFUSION INJURY seems to be exacerbated by activation of LPA receptors, indicating that the effect of LPA on cellular viability *in vivo* could be cell or context dependent³⁶.

LPA also exerts diverse vascular effects: it alters attachment of monocytes to vascular endothelial cells, it increases endothelial permeability (decreased barrier function), and it alters the contractility, proliferation and differentiation of vascular smooth-muscle cells, potentially contributing to its effects on blood pressure^{15,17,37-43}. These observations, together with the finding that LPA is an active ingredient of MILDLY OXIDIZED LOW DENSITY LIPOPROTEIN (LDL)⁴⁴⁻⁴⁵, indicate that LPA might contribute to ATHEROSCLEROSIS.

As well as its role as an extracellular messenger, LPA has a well-established 'house-keeping' role inside the cell, namely as a precursor in the biosynthesis of more complex phospholipids. In addition, intracellular LPA

Table 1 | Main biological activities of LPA

Effect	Cell type/remarks	Major signalling pathway/effector
Cell proliferation	Many normal and transformed cell types	G ₁ -RAS-ERK1/2
Cell survival		G ₁ -PI3K-AKT(PKB)
Cell migration (random and directed)	Diverse normal and transformed cell types	G ₁ -PI3K-TIAM1-RAC (together with RHOA and CDC42 pathways)
Tumour-cell invasion <i>in vitro</i>	Hepatoma, T lymphoma, carcinoma cells	
Wound healing <i>in vivo</i>	Skin, intestinal epithelium	
Morphological changes	Cell rounding, neurite retraction	G _{12/13} -RHO-GEF-RHOA
	Cell spreading, lamellipodia protrusion	G ₁ -PI3K-TIAM1-RAC
Inhibition/reversal of differentiation	Neuroblastoma cells (suppression of neurite outgrowth)	G _{12/13} -RHO-GEF-RHOA
	Astrocytes (reversal of stellation)	ERK and p38 MAPKs
	Vascular smooth-muscle cells (conversion to fibroblast morphology; loss of contractility)	
Contraction	Smooth-muscle cells, myofibroblasts	G _{12/13} -RHO-GEF-RHOA
Increased endothelial permeability	Micro- and macrovascular endothelial cells	G _{12/13} -RHO-GEF-RHOA
Inhibition of gap-junctional communication	Fibroblasts, hepatoma cells, epithelial cells	G _q -PLC

ERK, extracellular signal-regulated kinase; PI3K, phosphatidylinositol 3-kinase; MAPK, mitogen-activated protein kinase; PLC, phospholipase C.

can, at least *in vitro*, serve as a substrate for 'endophilin' — a cytosolic protein that can convert LPA into phosphatidic acid by addition of an acyl chain — and so could influence membrane curvature and endocytosis⁴⁶. Intriguingly, LPA has been reported to function as a high-affinity ligand for the transcription-factor peroxisome proliferating activating receptor- γ (PPAR γ)⁴⁷. Exogenous LPA can induce PPAR γ -mediated gene transcription, which supports the idea that this is a physiological role⁴⁷. PPAR γ normally binds fatty-acid derivatives and regulates genes that are involved in energy metabolism, cell differentiation, apoptosis and inflammation. As such, PPAR γ has a central role in ADIPOGENESIS and insulin sensitization, but it can also affect cell proliferation and differentiation in various malignancies. It will be necessary to determine how a charged phospholipid such as LPA would cross the plasma membrane intact (without degradation) and in sufficient quantity to activate a nuclear transcription factor. The relative contribution, if any, of PPAR γ activation to the physiological activities of extracellular LPA remains to be established.

G-protein signalling

The great variety of cellular and biological actions of LPA is explained by the fact that LPA receptors can couple to at least three distinct G proteins (G_s, G_i and G_{12/13}), which, in turn, feed into multiple effector systems^{2,11,32,33,48–54} (FIG. 2). LPA activates G_q and thereby stimulates PHOSPHOLIPASE C (PLC), with subsequent phosphatidylinositol-bisphosphate hydrolysis and generation of multiple second messengers leading to protein kinase C activation and changes in cytosolic calcium². LPA also activates G_i, which leads to at least three distinct signalling routes: inhibition of adenylyl cyclase with inhibition of cyclic AMP accumulation; stimulation of the mitogenic RAS-MAPK (mitogen-activated protein

kinase) cascade; and activation of phosphatidylinositol 3-kinase (PI3K), leading to activation of the guanosine diphosphate/guanosine triphosphate (GDP/GTP) exchange factor TIAM1 and the downstream RAC GTPase, as well as to activation of the AKT/PKB anti-apoptotic pathway^{2,48,51–54,56}. Finally, LPA activates G_{12/13}, leading to activation of the small GTPase RHOA, which drives cytoskeletal contraction and cell rounding^{50,55}. So, LPA not only signals via classic second messengers such as calcium, diacylglycerol and cAMP, but it also activates RAS- and RHO-family GTPases — the master switches that control cell proliferation, migration and morphogenesis. The RAS- and RHO-GTPases cycle between GDP- and GTP-bound states, with GTP binding being promoted by specific GDP/GTP exchange factors (GEFs); the GTP-bound forms can interact with various downstream effectors (such as protein kinases and scaffold proteins) and thereby alter cell behaviour.

By activating G_s, LPA triggers the RAS-mediated MAPK cascade and thereby promotes cell-cycle progression and cell survival^{2,51–53}. LPA-induced RAS activation presumably involves the RAS-specific GEF, SOS, and intermediate protein-tyrosine-kinase activity, but precisely how LPA activates RAS is still a matter of debate^{2,32}. In addition to activating RAS, LPA activates the RAC GTPase — a key regulator of the actin cytoskeleton, cell morphology and motility⁵⁴. LPA-induced RAC activation proceeds via a G_i-mediated pathway that involves enhanced PI3K activity and the RAC-specific GEF, TIAM1 (REF. 54). Interestingly, *Tiam1*-knockout mice are resistant to skin carcinogenesis, indicating that LPA-induced *Tiam1* activation could have a role in tumour initiation⁵⁷. Furthermore, LPA activates the RHOA GTPase via G_{12/13} and one or more specific RHO-GEFs, thereby inducing cytoskeletal contraction and cell rounding^{12,49,50}. So, the RHO-family GTPases RHOA and RAC are activated through two separate

ADIPOGENESIS
Development of fat cells (adipocytes) and formation of lipid bodies in adipocytes.

PHOSPHOLIPASE
An enzyme that cleaves phospholipids.

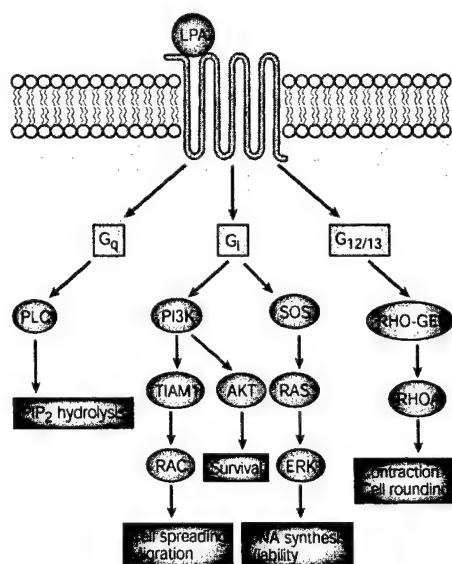


Figure 2 | Major LPA signalling pathways. LPA signals through its own G-protein-coupled receptors via at least three distinct classes of heterotrimeric G proteins — G_q , G_i and $G_{12/13}$ — leading to activation of multiple downstream effector pathways. Among the main LPA-induced signalling pathways are: G_q (or/and G_i)-mediated activation of phospholipase C (PLC), which leads to the hydrolysis of phosphatidylinositol-bisphosphate (PIP_2), with consequent calcium mobilization and protein kinase C (PKC) activation; G_i -mediated activation of the RAS-ERK pathway, leading to cell proliferation; G_i -mediated activation of the PI3K-AKT (also known as PKB) 'survival' pathway, which suppresses apoptosis; and activation of the RHO and RAC GTPases via specific exchange factors, RHO-GEF and TIAM1, which leads to cytoskeletal remodelling (contraction and spreading), shape changes and cell migration.

G-protein pathways — $G_{12/13}$ -RHO-GEF and G_i -PI3K-TIAM1 (REFS. 12,49,50,54). Their downstream effectors drive cell migration and invasion, but also impact on cell-cycle progression^{33,55}.

Finally, LPA induces cell-survival signalling through two pathways — via PI3K and AKT and through activation of the RAS/extracellular-signal-regulated kinase (ERK) pathway^{33,51}.

LPA receptors

Four mammalian cell-surface LPA receptors have been identified so far. The best known are LPA1, LPA2 and LPA3, which are all members of the so-called 'endothelial differentiation gene' (EDG) family of GPCRs and were formerly called EDG2, EDG4 and EDG7, respectively^{58–62}. Five additional members of the EDG-receptor subfamily encode related GPCRs that are specific for the bioactive lysophospholipid SPHINGOSINE-1-PHOSPHATE (S1P)⁶³. Recently, a fourth LPA receptor was identified (LPA4/GPR23/P2Y9), which shares no significant identity with the other LPA receptors⁶⁴. LPA4 is more closely related to the purinergic (P2Y) GPCR family, yet does not bind nucleotides.

SPHINGOSINE-1-PHOSPHATE
A small lipid that is similar to LPA, but with a sphingosine rather than a glycerol backbone.

LPA1 is the most widely expressed receptor, with high mRNA levels in the colon, small intestine, placenta, brain and heart, and more modest expression in the pancreas, ovary and prostate⁵⁸. Particularly high levels of LPA1 are present in the cerebral cortical ventricular zone during neurogenesis and in oligodendrocytes and Schwann cells in the adult. LPA2 and LPA3 have a more restricted distribution pattern compared with LPA1 (REFS. 58–62,65–66). Intriguingly, both LPA2 and LPA3 are aberrantly expressed in cancer cells, particularly in ovarian cancer cells, indicating a potential role in the pathophysiology of cancer^{18,66–70}. LPA4 seems to be expressed at very low levels in most human tissues, although significant levels are found in the ovary⁶⁴.

Genetic studies with Lpa-receptor-null mice have shown the importance of LPA-receptor function for normal development. *Lpa1*-null mice show a failure to suckle, potentially due to decreased olfaction, leading to weight loss and partial neonatal mortality⁷¹. Cell migration, rounding and proliferation in response to LPA is decreased in embryonic fibroblasts from *Lpa1*-null mice, but is not absent, consistent with redundant signalling from LPA receptors. *Lpa2*-null mice show no obvious phenotypic aberrations⁷² and, intriguingly, compound *Lpa1/Lpa2*-null mice do not show a phenotype different from that of *Lpa1*-null mice, except for a modest increase in frontal haematomas⁷². So, normal physiological functions either do not require LPA-receptor activation, or LPA3 and LPA4 (or other as yet unidentified LPA receptors) function redundantly. This also indicates that the main functions of the LPA receptors could become evident under pathophysiological conditions, such as wounding, inflammation or tumorigenesis, rather than during normal development. This further indicates that therapeutic modalities aimed at altering the function of specific LPA receptors might be well tolerated.

Nearly all mammalian cells, tissues and organs (except the liver) co-express several LPA-receptor subtypes of the EDG family, which strongly indicates that LPA receptors signal in a cooperative manner. However, which LPA receptor subtype couples to which G-protein-effector route(s) in a given cellular context is still not known. Heterologous expression studies have shown that each individual LPA receptor can mediate PLC activation, inhibition of cAMP accumulation and activation of the MAPK pathway, but there is considerable variation in the efficacy and potency that particular isoforms of LPA have for receptors, and the ability of receptors to link to particular downstream events. Indeed, in most systems LPA2 shows a higher affinity for LPA than for the other family members and couples more efficiently to production of neovascularizing factors (REF. 25 and E. Goetzl, personal communication). Similarly, LPA1 seems to be the main regulator of cellular motility^{54,71,73}.

Although a number of selective agonists and antagonists of the LPA receptors have been identified^{52,61,62,74–81}, their susceptibility to hydrolysis or the requirement for high concentrations of these inhibitors and lack of complete receptor specificity have hindered their use as therapeutic agents or probes of LPA-receptor function. The development of more selective inhibitors that cannot be

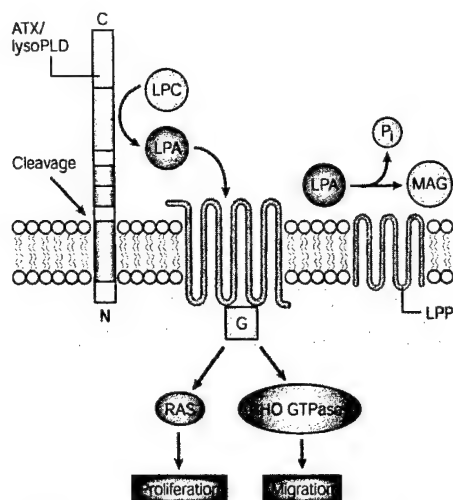


Figure 3 | Regulation of bioactive LPA. ATX/lysoPLD is synthesized as a large membrane-spanning glycoprotein that undergoes membrane-proximal proteolytic cleavage (not necessarily at the plasma membrane) to yield a secreted enzyme. Soluble ATX/lysoPLD hydrolyses LPC (and other lysophospholipids), which is abundantly present in the circulation, to generate LPA. Newly produced LPA acts on its own G-protein-coupled receptors to evoke its biological effects, including RAS-mediated cell proliferation and RHO/RAC-regulated cell migration. Excess LPA is converted into monoacylglycerol (MAG) by membrane-bound lipid phosphatases (LPPs) that remove the phosphate group from LPA.

hydrolysed is eagerly awaited. Nevertheless, the development of these molecules bodes well for the eventual ability to selectively target LPA receptors and functions.

The structural basis of receptor selectivity of LPA is beginning to be elucidated^{52,61,62,74–82}. Molecular modelling could soon contribute to the development of receptor-selective agonists and antagonists that will both elucidate receptor function and serve as therapeutic mediators. For example, a single amino acid glutamine/glutamate exchange in transmembrane helix 3 between LPA1 and the S1P receptor S1P1 is sufficient to confer the ability to respond to the heterologous ligand⁷⁷, indicating that the binding specificity is probably conferred by modest structural changes in the receptors.

A number of important questions remain unanswered. For example, what is the nature of the signalling pathways that are activated by each individual LPA receptor, what are the downstream signal transducers and do distinct LPA receptors signal in a cooperative manner? Are the effects of activation of specific LPA receptors context dependent and altered by the particular intracellular machinery in the responding cell or by the cellular environment? How the diverse biochemical events activated by LPA are integrated into functional outcomes has remained particularly elusive. Future microarray studies, using specific LPA-receptor antagonists and agonists or cells in which LPA-receptor expression is selectively

altered, might shed light on the unique gene-expression pattern that is induced by each LPA-receptor subtype in a given cellular context.

Extracellular metabolism of LPA

Physiologically significant concentrations of LPA are found not only in serum, but also in malignant effusions, saliva, follicular fluid, seminal plasma and in mildly oxidized LDL. In serum, and presumably in other body fluids, the main LPA-binding proteins/carriers are albumin and gelsolin^{83–84}. One of the long-standing challenges has been to understand how bioactive LPA is produced and its level regulated in the cellular microenvironment. Emerging evidence indicates that bioactive LPA is generated extracellularly, rather than inside the cell, with subsequent secretion or release^{85–87}. Indeed, a series of secreted and transmembrane ecto-enzymes are crucial for the production and metabolism of extracellular LPA^{86–93} (FIGS 1,3). Steady-state plasma LPA levels are normally low (less than 100 nM)^{86,87,94,95}, representing the equilibrium between production, degradation and clearance. Unidentified factors in plasma and seminal fluid seem to inhibit LPA activity^{87,96}. So, the effective levels of bioactive LPA in plasma are below those able to induce full activation of LPA receptors (usually observed at 100–200 nM LPA). Nevertheless, plasma contains both the enzymes and the lipid substrates that are required for LPA production. Incubation of plasma at 37°C, for example, results in an increase in LPA levels to those necessary to activate LPA receptors⁸⁶. The mechanisms that restrict LPA production in plasma, as well as the triggers that increase LPA production during pathophysiological states, remain unclear. LPA is probably produced locally — following injury or stress such as wound healing — at the interface between cells and the interstitial fluid, and then migrates to plasma for degradation or clearance.

Whereas plasma levels of LPA are low, its concentration in serum is much higher at several micrometers (μM). LPA is produced following platelet activation^{85,97}, but the amount of LPA released is insufficient to explain LPA levels in serum^{86,87}. Furthermore, the fatty acyl chain composition of LPA produced by platelets^{85,97} differs from that in plasma or serum, which contains higher levels of LPA enriched with unsaturated fatty acids⁹⁷. This indicates that different processes regulate production and degradation of LPA by platelets and in plasma, or following activation of the coagulation cascade.

LPA production

LPA can be produced by the sequential removal of a fatty acyl chain from phosphatidic acid by phospholipase A1 (PLA1) or phospholipase A2 (PLA2), or by the removal of choline from membrane phosphatidylcholine by ATX/lysoPLD (lyso phospholipase D)^{86,87}. PLA1 releases fatty acids from the sn-1 position of membrane phospholipids (FIG. 1) that, when converted to LPA, produce sn-2 and polyunsaturated LPA isoforms that are selectively active on the LPA3 receptor^{61,81}. Type-II secretory

PLASMA
Fluid that is present in blood
in vivo.

Box 1 | Properties of ATX/lysoPLD

- Originally discovered as an autocrine motility factor (125 kDa) that is secreted by human melanoma cells and subsequently characterized as a transmembrane ecto-nucleotide phosphodiesterase (also termed NPP2).
- The soluble form is identical to plasma lysoPLD, which generates LPA from more complex lysophospholipids such as lysophosphatidylcholine (LPC).
- Widely expressed (highest expression in the brain, lung, ovary, kidney, intestine and testis).
- Upregulated in several cancers (including non-small-cell lung carcinoma, renal-cell carcinoma, mammary carcinoma, neuroblastoma and hepatocellular carcinoma).
- Upregulated by peptide growth factors (such as bFGF and BMP2), retinoic acid and WNT signalling (in mammary epithelial cells).
- Stimulates tumour aggressiveness, metastasis and angiogenesis in nude mice.
- ATX/lysoPLD-induced cell motility and proliferation are mediated by newly produced LPA.

phospholipase A2 (sPLA2), which cleaves fatty acyl chains from the sn-2 site (FIG. 1), has a limited ability to hydrolyse lipids in intact cell membranes, potentially contributing to the low levels of LPA in plasma. sPLA2 selectively hydrolyses lipids that are present in damaged membranes, membranes of activated cells, or microvesicles such as those released during apoptosis or that are produced by cancer cells^{98,99}. Microvesicle numbers are particularly high in malignant fluids such as ASCITES, potentially contributing to the aberrant production of LPA in cancer patients^{69,98,99,115–117}.

The recent discovery that LPA is generated from lysophospholipids, particularly from lysophosphatidylcholine, by the previously enigmatic ATX/lysoPLD ecto-phosphodiesterase^{90,91} (BOX 1; FIG. 3) — implicated in cell motility and tumour progression^{100,102} — has shed light on how LPA is produced in the extracellular milieu. It has also provided impetus into studies of the role of ATX/lysoPLD and LPA in the metastatic cascade.

ATX was originally identified as an 'autocrine motility factor' secreted by melanoma cells¹⁰⁰ and was subsequently found to belong to the family of ecto-nucleotide phosphodiesterases (NPPs), which are capable of hydrolysing phosphodiester and pyrophosphate bonds that are typically found in ATP and ADP¹⁰¹. Therefore, ATX was previously thought to act via nucleotide (purinergic) receptor signalling. However, new work convincingly shows that ATX has lysoPLD activity and that the biological effects of ATX/lysoPLD can be attributed to the production of LPA and, potentially, S1P^{90,91,103–105} (FIG. 3). Unexpectedly, it seems that ATX is a unique lysoPLD, in that its family members (NPP1 and NPP3) lack a similar phospholipase function¹⁰⁵.

ATX/lysoPLD is a transmembrane protein with a very short amino-terminal region, a single transmembrane domain, two cysteine-rich somatomedin-B-like domains

— possibly involved in homodimerization — and a large catalytic ecto-domain¹⁰¹. Soluble ATX/lysoPLD is derived from the membrane-bound form by proteolytic cleavage, but details of ATX/lysoPLD biosynthesis and processing are still largely unknown. ATX/lysoPLD is widely expressed, with the highest mRNA levels in the brain, ovary, lung, intestine and kidney, and it is upregulated by certain peptide growth factors^{106,107}. Targeted deletion and transgenic overexpression of ATX/lysoPLD in specific tissues should provide important insights into its (patho)physiological functions.

Outstanding questions concern the regulation of ATX/lysoPLD expression, activity and processing. Where is ATX/lysoPLD localized in cells? How and where is its proteolytic cleavage regulated? Does full-length ATX/lysoPLD exist on the cell surface? Is full-length ATX/lysoPLD catalytically active? What is the source of ATX/lysoPLD in plasma? How does ATX/lysoPLD participate in the development of pathophysiological states? Finally, how is its lipid substrate(s) locally produced? With regard to this last question, the main physiological substrate for ATX/lysoPLD is lysophosphatidylcholine (LPC) (FIG. 1). LPC is secreted by the liver and is abundantly present in plasma, where it is predominantly bound to albumin and, to a lesser extent, lipoproteins¹⁰⁸. LPC is also found in the supernatant of cultured cells, presumably as a constituent of microvesicles that have been shed from the plasma membrane⁹². Interestingly, microvesicle shedding has been implicated in the metastatic cascade, with malignant body fluids (for example, ascites) containing large amounts of microvesicles^{98,99}. So, ATX/lysoPLD could convert microvesicle-associated LPC into bioactive LPA, which would provide an explanation for the link between microvesicle shedding and metastasis.

The original identification of secreted ATX/lysoPLD as a tumour-motility factor indicates a link between ATX/lysoPLD and cancer, and strengthens the evidence for a role for LPA in the initiation and progression of cancer. Indeed, *ATX/lysoPLD* mRNA is upregulated in several human cancers, particularly melanoma, renal-cell carcinoma and glioma, and studies in nude mice have shown that ATX/lysoPLD enhances tumour aggressiveness¹⁰⁹. Specifically, *Atx*-transfected, *Ras*-transformed NIH-3T3 cells are more invasive, tumorigenic and metastatic than *Ras*-transformed control cells. Further, the metastatic capability of breast cancer cells correlates with their ATX/lysoPLD levels¹¹⁰. These observations are compatible with the main activity of ATX/lysoPLD being due to production of LPA and its effects on protease production, cell motility, chemotaxis and invasion. Furthermore, ATX/lysoPLD can promote angiogenesis both *in vitro* and *in vivo*¹⁰².

So, by generating LPA (and possibly other bioactive lysophospholipids), ATX/lysoPLD could contribute to tumour progression by providing an invasive and vasculogenic microenvironment for tumour cells. As a secreted or cell-surface enzyme, ATX/lysoPLD is a highly attractive pharmacological target for therapy and might be of diagnostic value.

ASCITES FLUID
Fluid that accumulates in the peritoneal cavity of ovarian cancer patients and occasionally in patients with other diseases, such as liver failure.

LPA inactivation

Production of bioactive LPA by ATX/lysoPLD is only half of the story: obviously, LPA accumulation must be counterbalanced by inactivation mechanisms. One possibility is that ATX/lysoPLD activity is tightly controlled by as-yet-unidentified cofactors or binding proteins. An alternative or additional mechanism is provided by inactivation of LPA itself. Indeed, exogenous LPA is rapidly dephosphorylated to yield biologically inactive monoacylglycerol. Recent advances have revealed that a family of lipid phosphate phosphohydrolases (LPPs), comprising at least four members, is responsible for the dephosphorylation of LPA and can therefore attenuate LPA signalling^{79,88,89,93}. The LPPs are integral membrane ecto-enzymes, with six putative transmembrane domains⁹¹. Overexpression of LPPs in ovarian cancer cells decreases colony formation, increases apoptosis and decreases tumour growth *in vitro* and *in vivo*⁹³. Interestingly, LPP activity can be increased by gonadotropin-releasing hormone analogues through recruitment of LPPs to the cell membrane⁸⁸. So, LPA signal duration and strength is likely to depend, at least in part, on the expression level of LPPs, which are decreased in ovarian cancer¹¹¹, and their membrane localization relative to the LPA receptors. Pharmacological manipulation to increase LPP activity is an intriguing approach for cancer therapy.

LPA in the pathophysiology of cancer

The first indication that LPA could contribute to tumorigenesis came from studies showing that LPA increases motility and invasiveness of cells^{3,9,21,22,24}. Studies from our groups, combined with the observation that ATX/lysoPLD mediates its effects through the production of LPA, have implicated LPA in the pathophysiology of ovarian cancer, and several studies have indicated a role for LPA in the initiation or progression of prostate, breast, melanoma, head and neck, bowel, thyroid and other cancers^{5,23–25,52,66–70,73,99,101–104,109,111,112}.

Ascites fluid in ovarian cancer patients provides a window on the cellular environment of the tumour, as well as on growth factors that are produced by tumour cells. Ascites fluid is a potent mitogen for ovarian cancer cells *in vitro* and *in vivo*^{113–114}. A significant portion of this activity is mediated by LPA, which is present in ascites fluid at between 1 and 80 μM , exceeding levels required to optimally activate LPA receptors^{70,95,115–117}. LPA is not produced at significant levels by normal ovarian epithelial cells, whereas ovarian cancer cells produce increased levels of LPA^{69,118}. Prostate cancer cells also produce high levels of LPA and respond to LPA in an autocrine loop¹¹². Surprisingly, LPA itself, as well as phorbol esters, are sufficient to increase production of LPA by some ovarian cancer cell lines, indicating the presence of autocrine networks^{69,118}. Ovarian cancer cells do not express unusually high levels of ATX/lysoPLD mRNA; however, ATX/lysoPLD protein levels are increased in most ovarian cancers. Furthermore, as ovarian cancer patients present with large tumour masses, low-level production of ATX/lysoPLD or LPA by ovarian cancer cells might be

sufficient to result in increased levels of LPA in ascites. Indeed, ATX/lysoPLD activity is increased in most ovarian cancer ascites. By contrast, LPP1 mRNA levels are consistently decreased in ovarian cancer samples¹¹⁰, indicating that LPA inactivation might be decreased, which would also contribute to the increased levels of LPA in ascites.

LPA2 and LPA3 are overexpressed by a significant fraction of ovarian cancer cells, contributing to the responsiveness of ovarian cancer cells to LPA^{66–69}. LPA4 levels are particularly high in normal ovary⁶⁵, potentially contributing to the effects of LPA on ovarian cancer cells. Colorectal carcinoma cell lines show significant expression of LPA1 mRNA and respond to LPA by cell migration and production of angiogenic factors⁷³. It has been suggested that LPA2 overexpression has a role in the pathogenesis of thyroid cancer⁷⁰. LPA3 was originally cloned from prostate cancer cells, concordant with the ability of LPA to induce autocrine proliferation of prostate cancer cells^{62,112}.

Lysophospholipids as tumour markers

The increased levels of LPA and vesicles in ascites from ovarian cancer patients indicated that, if LPA migrates from the peritoneal cavity into the circulation, it could be an early diagnostic marker, a prognostic indicator or an indicator of response to therapy. LPA levels are consistently higher in ascites samples than in matched plasma samples, which is compatible with this hypothesis⁶⁹. Preliminary studies using purification followed by GAS CHROMATOGRAPHY (GC) indicated that levels of LPA or particular isoforms of LPA were increased in the plasma of approximately 90% of ovarian cancer patients^{94,119}. LPA levels were not increased in samples from patients with breast cancer or leukaemia, but were increased in patients with myeloma, endometrial cancer and cervical cancer, as well as patients on renal dialysis^{94,119–121}. A similar approach showed aberrations in particular LPC isoforms in plasma from ovarian cancer patients¹²². More recent studies using a mass-spectrometry approach failed to detect increased levels of LPA in plasma from ovarian cancer patients or alterations in levels of particular isoforms⁹⁵, although even this finding is controversial^{12,124}. An analysis of ATX/lysoPLD activity failed to detect aberrant activity in blood samples from ovarian cancer patients¹²⁵. The discrepancy between the results might represent differences in the technologies used or in the way in which the samples were collected or handled. It therefore remains possible that assessment of particular isoforms of the many different lysophospholipids present in plasma and ascites might provide diagnostic or prognostic information. Indeed, based on different backbones, fatty-acyl-chain linkage, location, length and saturation, and phosphate modification, several hundred different lysophospholipids could be present in plasma and ascites.

Prospects for the future

Almost half of all drugs in current use target members of the GPCR family, making LPA receptors attractive

LPPS
(Lipid phosphate phosphohydrolases). Cleave phosphate from LPA, sphingosine-1-phosphate, ceramide-1-phosphate and phosphatidic acid.

GAS CHROMATOGRAPHY
A method that is used to separate and identify small molecules.

targets for therapeutic development. As described above, LPA receptors are broadly expressed, including in the brain and vasculature, which has led to concerns about toxicity of inhibitors of LPA production or action. However, as compound *Lpa1/Lpa2*-null mice are viable and do not show marked functional aberrations in adult mice, it might be possible to target specific LPA receptors in tumour cells without undue systemic toxicity. LPA3 is particularly appealing as its expression is restricted and it is aberrantly expressed in multiple cancer lineages. Structure-function analysis, molecular modelling and studies of receptor structure are already contributing to the development of novel receptor-selective agonists and antagonists. However, as yet, therapeutically relevant LPA agonists and antagonists have not been developed. In addition to inhibition of receptor activation, LPA production and degradation could prove attractive targets for

therapy. Both ATX/lysoPLD and LPPs are extracellular enzymes and are therefore readily available targets for therapy. In addition to its direct actions on tumour cells, LPA also prevents intestinal damage (epithelial apoptosis) in irradiated or chemotherapy-treated mice¹²⁶. The latter finding indicates that diets that are rich in LPA or therapeutic modulation of LPA activity could be effective in reducing intestinal damage in patients undergoing cancer therapy. The development of selective LPA-receptor agonists and antagonists, as well as the identification of the pathways regulating LPA production and action, indicates that therapeutic approaches targeting the LPA cascade might be a realistic addition to the treatment of malignant disease in the near future. We look forward to the future, when the role of LPA and related lysophospholipids in the physiology, pathophysiology and management of cancer and other diseases is elucidated.

1. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100**, 57-70 (2000).
2. van Corven, E. J., Groenink, A., Jalink, K., Eichholtz, T. & Moolenaar, W. H. Lysophosphatidate-induced cell proliferation: identification and dissection of signalling pathways mediated by G proteins. *Cell* **59**, 45-54 (1989).
3. **Seminal observation that LPA has growth-factor-like activities, and signals in a strictly G-protein-dependent manner.**
4. Imamura, F. et al. Induction of *in vitro* tumour cell invasion of cellular monolayers by lysophosphatidic acid or phospholipase D. *Biochem. Biophys. Res. Commun.* **193**, 497-503 (1993).
5. Jalink, K., Hordijk, P. L. & Moolenaar, W. H. Growth factor-like effects of lysophosphatidic acid, a novel lipid mediator. *Biochim. Biophys. Acta* **1196**, 185-196 (1994).
6. Xu, Y., Fang, X. J., Casey, G. & Mills, G. B. Lysophospholipids activate ovarian and breast cancer cells. *Biochem. J.* **309**, 933-940 (1995).
7. Xu, Y., Casey, G. & Mills, G. B. Effect of lysophospholipids on signaling in the human Jurkat T cell line. *J. Cell Physiol.* **163**, 441-450 (1995).
8. Levine, J. S., Koh, J. S., Triaca, V. & Lieberthal, W. Lysophosphatidic acid: a novel growth and survival factor for renal proximal tubular cells. *Am. J. Physiol.* **273**, F575-F585 (1997).
9. Altun-Gultekin, Z. F. et al. Activation of Rho-dependent cell spreading and focal adhesion biogenesis by the v-Crk adaptor protein. *Mol. Cell Biol.* **18**, 3044-3058 (1998).
10. Stam, J. C., Michiels, F., van der Kammen, R. A., Moolenaar, W. H. & Collard, J. G. Invasion of T-lymphoma cells: cooperation between Rho family GTPases and lysophospholipid receptor signaling. *EMBO J.* **17**, 4066-4074 (1998).
11. Weiner, J. A. & Chun, J. Schwann cell survival mediated by the signaling phospholipid lysophosphatidic acid. *Proc. Natl Acad. Sci. USA* **96**, 5233-5238 (1999).
12. Jalink, K., Eichholtz, T., Postma, F. R., van Corven, E. J. & Moolenaar, W. H. Lysophosphatidic acid induces neuronal shape changes via a novel, receptor-mediated signaling pathway: similarity to thrombin action. *Cell Growth Differ.* **4**, 247-255 (1993).
13. **First demonstration that LPA induces neurite retraction in neuroblastoma cells.**
14. Jalink, K., van Corven, E. J., Hengeveld, T., Morii, N., Narumiya, S. & Moolenaar, W. H. Inhibition of lysophosphatidate- and thrombin-induced neurite retraction and neuronal cell rounding by ADP-ribosylation of the small GTP-binding protein Rho. *J. Cell Biol.* **126**, 801-810 (1994).
15. **First paper indicating a role for RHOA in LPA-induced 'dedifferentiation' of neuroblastoma cells, independently of classic second messengers.**
16. Hill, C. S., Oh, S. Y., Schmidt, S. A., Clark, K. J. & Murray, A. W. Lysophosphatidic acid inhibits gap-junctional communication and stimulates phosphorylation of connexin-43 in WB cells: possible involvement of the mitogen-activated protein kinase cascade. *Biochem. J.* **303**, 475-479 (1994).
17. Tigyi, G. et al. Lysophosphatidic acid-induced neurite retraction in PC12 cells: control by phosphoinositide- Ca^{2+} signaling and Rho. *J. Neurochem.* **66**, 537-548 (1996).
18. Schulze, C., Smales, C., Rubin, L. L. & Staddon, J. M. Lysophosphatidic acid increases tight junction permeability in cultured brain endothelial cells. *J. Neurochem.* **68**, 991-1000 (1997).
19. Postma, F. R. et al. Acute loss of cell-cell communication caused by G protein-coupled receptors: a critical role for c-Src. *J. Cell Biol.* **140**, 1199-1209 (1998).
20. van Nieuw Amerongen, G. P., Vermeer, M. A. & van Hinsbergh, V. W. Role of RhoA and Rho kinase in lysophosphatidic acid-induced endothelial barrier dysfunction. *Arterioscler. Thromb. Vasc. Biol.* **20**, E127-E133 (2000).
21. Pustilnik, T. B. et al. Lysophosphatidic acid induces urokinase secretion by ovarian cancer cells. *Clin. Cancer Res.* **5**, 3704-3710 (1999).
22. Palmesthofer, A., Robson, S. C. & Nehls, V. Lysophosphatidic acid activates nuclear factor kappa B and induces proinflammatory gene expression in endothelial cells. *Thromb. Haemost.* **82**, 1532-1537 (1999).
23. Schwartz, B. M. et al. Lysophospholipids increase interleukin-8 expression in ovarian cancer cells. *Gynecol. Oncol.* **81**, 291-300 (2001).
24. Fishman, D. A., Liu, Y., Ellerbroek, S. M. & Stack, M. S. Lysophosphatidic acid promotes matrix metalloproteinase (MMP) activation and MMP-dependent invasion in ovarian cancer cells. *Cancer Res.* **61**, 3194-3199 (2001).
25. Zheng, Y., Kong, Y. & Goetzl, E. J. Lysophosphatidic acid receptor-selective effects on Jurkat T cell migration through a Matrigel model basement membrane. *J. Immunol.* **166**, 2317-2322 (2001).
26. Hu, Y. L. et al. Lysophosphatidic acid induction of vascular endothelial growth factor expression in human ovarian cancer cells. *J. Natl Cancer Inst.* **93**, 762-768 (2001).
27. Gschwind, A., Hart, S., Fischer, O. M. & Ullrich, A. TACE cleavage of proamphiregulin regulates GPCR-induced proliferation and motility of cancer cells. *EMBO J.* **22**, 2411-2421 (2003).
28. **Novel mechanism for transactivation of tyrosine-kinase receptors by G-protein-coupled receptors.**
29. Fang, X. et al. Mechanisms of lysophosphatidic acid-induced cytokine production in ovarian cancer cells. *J. Biol. Chem.* (in press).
30. Sturm, A., Sudermann, T., Schulte, K. M., Goebel, H. & Dignass, A. U. Modulation of intestinal epithelial wound healing *in vitro* and *in vivo* by lysophosphatidic acid. *Gastroenterology* **117**, 368-377 (1999).
31. Lee, H., Goetzl, E. J. & An, S. Lysophosphatidic acid and sphingosine 1-phosphate stimulate endothelial cell wound healing. *Am. J. Physiol. Cell Physiol.* **278**, C612-C618 (2000).
32. Watsky, M. A., Griffith, M., Wang, D. A. & Tigyi, G. J. Phospholipid growth factors and corneal wound healing. *Ann. NY Acad. Sci.* **905**, 142-158 (2000).
33. Balazs, L., Okolichany, J., Ferrebee, M., Tolley, B. & Tigyi, G. Topical application of the phospholipid growth factor lysophosphatidic acid promotes wound healing *in vivo*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **280**, R466-R472 (2001).
34. Daub, H., Weiss, F. U., Wallasch, C. & Ullrich, A. Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors. *Nature* **379**, 557-560 (1996).
35. **Demonstration of cross-talk between G-protein-coupled receptors and receptor protein tyrosine kinases.**
36. Prenzel, N. et al. EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. *Nature* **402**, 884-888 (1999).
37. Hordijk, P. L., Verlaan, I., van Corven, E. J. & Moolenaar, W. H. Protein tyrosine phosphorylation induced by lysophosphatidic acid in Rat-1 fibroblasts. Evidence that phosphorylation of map kinase is mediated by the G-p21ras pathway. *J. Biol. Chem.* **269**, 645-651 (1994).
38. Kumagai, N., Mori, N., Fujisawa, K., Nemoto, Y. & Narumiya, S. ADP-ribosylation of rho p21 inhibits lysophosphatidic acid-induced protein tyrosine phosphorylation and phosphatidylinositol 3-kinase activation. *J. Biol. Chem.* **268**, 24535-24538 (1993).
39. Holtsberg, F. W. et al. Lysophosphatidic acid induces necrosis and apoptosis in hippocampal neurons. *J. Neurochem.* **70**, 66-76 (1998).
40. Holtsberg, F. W. et al. Lysophosphatidic acid and apoptosis of nerve growth factor-differentiated PC12 cells. *J. Neurosci. Res.* **53**, 685-696 (1998).
41. Okusa, M. D. et al. Selective blockade of lysophosphatidic acid LPA3 receptors reduces murine renal ischemia-reperfusion injury. *Am. J. Physiol. Renal Physiol.* **277**, May 2003 [epub ahead of print].
42. **Use of receptor-selective LPA agonists and antagonists *in vivo* to define the function and role of particular LPA receptors in ischaemia reperfusion.**
43. Tokumura, A. et al. Lysophosphatidic acids induce proliferation of cultured vascular smooth muscle cells from rat aorta. *Am. J. Physiol.* **267**, C204-C210 (1994).
44. Tokumura, A., Yotsutomo, T., Masuda, Y. & Tanaka, S. Vasopressor effect of lysophosphatidic acid on spontaneously hypertensive rats and Wistar Kyoto rats. *Res. Commun. Mol. Pathol. Pharmacol.* **90**, 96-102 (1995).
45. Ceruti, D. R. et al. Lysophosphatidic acid and EGF stimulate mitogenesis in human airway smooth muscle cells. *Am. J. Physiol.* **273**, L10-L15 (1997).
46. Toews, M. L., Ushiova, E. E. & Schultz, H. D. Lysophosphatidic acid enhances contractility of isolated airway smooth muscle. *J. Appl. Physiol.* **83**, 1216-1222 (1997).
47. Rizza, C. et al. Lysophosphatidic acid as a regulator of endothelial/leukocyte interaction. *Lab. Invest.* **79**, 1227-1235 (1999).
48. Scala, R., Pruefer, D. & Lefer, A. M. A novel lysophosphatidic acid analog, LXR-1035, inhibits leukocyte-endothelium interaction via inhibition of cell adhesion molecules. *J. Leukoc. Biol.* **67**, 26-33 (2000).
49. Hayashi, K. et al. Phenotypic modulation of vascular smooth muscle cells induced by unsaturated lysophosphatidic acids. *Circ. Res.* **89**, 251-258 (2001).

44. Natarajan, V., Scribner, W. M., Hart, C. M. & Parthasarathy, S. Oxidized low density lipoprotein-mediated activation of phospholipase D in smooth muscle cells: a possible role in cell proliferation and atherogenesis. *J. Lipid Res.* **36**, 2005-2016 (1995).
45. Sless, W. et al. Lysophosphatidic acid mediates the rapid activation of platelets and endothelial cells by mildly oxidized low density lipoprotein and accumulates in human atherosclerotic lesions. *Proc. Natl Acad. Sci. USA* **96**, 6931-6936 (1999).
46. Schmidt, A. et al. Endophilin I mediates synaptic vesicle formation by transfer of arachidonate to lysophosphatidic acid. *Nature* **401**, 133-141 (1999).
47. McIntyre, T. M. et al. Identification of an intracellular receptor for lysophosphatidic acid (LPA): LPA is a transcellular PPARgamma agonist. *Proc. Natl Acad. Sci. USA* **100**, 131-136 (2003).
48. van Corven, E. J., Hordijk, P. L., Medema, R. H., Bos, J. L. & Moolenaar, W. H. Pertussis toxin-sensitive activation of p21ras by G protein-coupled receptor agonists in fibroblasts. *Proc. Natl Acad. Sci. USA* **90**, 1257-1261 (1993).
49. van Dijk, M. C. et al. Exogenous phospholipase D generates lysophosphatidic acid and activates Ras, Rho and Ca²⁺ signaling pathways. *Curr. Biol.* **8**, 386-392 (1998).
50. Kranenburg, O. et al. Activation of RhoA by lysophosphatidic acid and G_{12/13} subunits in neuronal cells: induction of neurite retraction. *Mol. Biol. Cell* **10**, 1851-1857 (1999).
51. Fang, X. et al. Lysophosphatidic acid prevents apoptosis in fibroblasts via G(i)-protein-mediated activation of mitogen-activated protein kinase. *Biochem. J.* **352**, 135-143 (2000).
52. Fang, X. et al. Lysophospholipid growth factors in the initiation, progression, metastases, and management of ovarian cancer. *Ann. NY Acad. Sci.* **905**, 188-208 (2000).
53. Kranenburg, O. & Moolenaar, W. H. Ras-MAP kinase signaling by lysophosphatidic acid and other G protein-coupled receptor agonists. *Oncogene* **20**, 1540-1546 (2001).
54. Van Leeuwen, F. N. et al. Rac activation by lysophosphatidic acid LPA1 receptors through the guanine nucleotide exchange factor TIAM1. *J. Biol. Chem.* **278**, 400-406 (2003).
55. Etienne-Manneville, S. & Hall, A. Rho GTPases in cell biology. *Nature* **420**, 629-635 (2002).
56. Takeda, H. et al. PI 3-kinase gamma and protein kinase C-zeta mediate RAS-independent activation of MAP kinase by a G protein-coupled receptor. *EMBO J.* **18**, 386-395 (1999).
57. Mallin, A. et al. Mice deficient in the Rac activator TIAM1 are resistant to Ras-induced skin tumours. *Nature* **417**, 867-871 (2002).
58. Hecht, J. H., Weiner, J. A., Post, S. R. & Chun, J. Ventricular zone gene-1 (vzg-1) encodes a lysophosphatidic acid receptor expressed in neurogenic regions of the developing cerebral cortex. *J. Cell. Biol.* **135**, 1071-1083 (1996).
59. An, S., Dickens, M. A., Bleu, T., Hallmark, O. G. & Goetzl, E. J. Molecular cloning of the human Edg2 protein and its identification as a functional cellular receptor for lysophosphatidic acid. *Biochem. Biophys. Res. Commun.* **231**, 619-622 (1997).
60. An, S., Bleu, T., Hallmark, O. G. & Goetzl, E. J. Characterization of a novel subtype of human G protein-coupled receptor for lysophosphatidic acid. *J. Biol. Chem.* **273**, 7906-7910 (1998).
61. Bandoh, K. et al. Molecular cloning and characterization of a novel human G-protein-coupled receptor, EDG7, for lysophosphatidic acid. *J. Biol. Chem.* **274**, 27716-27785 (1999).
62. Im, D. S. et al. Molecular cloning and characterization of a lysophosphatidic acid receptor, Edg-7, expressed in prostate. *Mol. Pharmacol.* **57**, 753-759 (2000).
63. Hla, T., Lee, M. J., Ancellin, N., Paik, J. H. & Kluk, M. J. Lysophospholipids—receptor revelations. *Science* **294**, 1875-1878 (2001).
64. Noguchi, K., Ishii, S. & Shimizu, T. Identification of p2y9/GPR23 as a novel G protein-coupled receptor for lysophosphatidic acid, structurally distant from the Edg family. *J. Biol. Chem.* **30** Apr 2003 [pub ahead of print].
65. Contos, J. J. & Chun, J. The mouse lpA3/Edg7 lysophosphatidic acid receptor gene: genomic structure, chromosomal localization, and expression pattern. *Gene* **267**, 243-253 (2001).
66. Fang, X. et al. Lysophosphatidic acid is a bioactive mediator in ovarian cancer. *Biochim. Biophys. Acta* **1582**, 257-264 (2002).
67. Goetzl, E. J. et al. Distinctive expression and functions of the type 4 endothelial differentiation gene-encoded G protein-coupled receptor for lysophosphatidic acid in ovarian cancer. *Cancer Res.* **59**, 5370-5375 (1999).
68. Furui, T. et al. Overexpression of edg-2/vzg-1 induces apoptosis and anoikis in ovarian cancer cells in a lysophosphatidic acid-independent manner. *Clin. Cancer Res.* **8**, 4308-4318 (1999).
69. Eder, A. M., Sasagawa, T., Mao, M., Aoki, J. & Mills, G. B. Constitutive and lysophosphatidic acid (LPA)-induced LPA production: role of phospholipase D and phospholipase A2. *Clin. Cancer Res.* **6**, 2482-2491 (2000).
70. Schulte, K. M., Beyer, A., Kohrer, K., Oberhauser, S. & Rohrer, H. D. Lysophosphatidic acid, a novel lipid growth factor for human thyroid cells: over-expression of the high-affinity receptor edg4 in differentiated thyroid cancer. *Int. J. Cancer* **92**, 249-256 (2001).
71. Contos, J. J., Fukushima, N., Weiner, J. A. & Kauschal, D. J. Requirement for the LPA1 lysophosphatidic acid receptor gene in normal suckling behavior. *Proc. Natl Acad. Sci. USA* **97**, 13384-13389 (2000).
72. Contos, J. J. et al. Characterization of lpA2 (Edg4) and lpA1 (lpA2) (Edg2/Edg4) lysophosphatidic acid receptor knockout mice: signaling deficits without obvious phenotypic abnormality attributable to lpA2. *Mol. Cell Biol.* **22**, 6921-6929 (2002).
73. Shida, D. et al. Lysophosphatidic acid (LPA) enhances the metastatic potential of human colon carcinoma DLD1 cells through LPA1. *Cancer Res.* **63**, 1706-1711 (2003).
74. Fischer, D. J. et al. Naturally occurring analogs of lysophosphatidic acid elicit different cellular responses through selective activation of multiple receptor subtypes. *Mol. Pharmacol.* **54**, 979-988 (1998).
75. Erickson, J. R., Espinal, G. & Mills, G. B. Analysis of the EDG2 receptor based on the structure/activity relationship of LPA. *Ann. NY Acad. Sci.* **905**, 279-281 (2000).
76. Fischer, D. J. et al. Short-chain phosphatidates are subtype-selective antagonists of lysophosphatidic acid receptors. *Mol. Pharmacol.* **60**, 776-784 (2001).
77. Wang, D. A. et al. A single amino acid determines lysophospholipid specificity of the S1P1 (EDG1) and LPA1 (EDG2) phospholipid growth factor receptors. *J. Biol. Chem.* **276**, 49213-49220 (2001).
78. Heise, C. E. et al. Activity of 2-substituted lysophosphatidic acid (LPA) analogs at LPA receptors: discovery of a LPA1/LPA3 receptor antagonist. *Mol. Pharmacol.* **60**, 173-180 (2001).
79. Hooks, S. B. et al. Lysophosphatidic acid-induced mitogenesis is regulated by lipid phosphate phosphatases and is Edg-receptor independent. *J. Biol. Chem.* **276**, 4611-4621 (2001).
80. Hasegawa, Y. et al. Identification of a phosphothionate analogue of lysophosphatidic acid (LPA) as a selective agonist of the LPA3 receptor. *J. Biol. Chem.* **278**, 11962-11969 (2003).
81. Bandoh, K. et al. Lysophosphatidic acid (LPA) receptors of the EDG family are differentially activated by LPA species. Structure-activity relationship of cloned LPA receptors. *FEBS Lett.* **478**, 159-165 (2000).
82. Vrag, T. et al. Fatty alcohol phosphates are subtype-selective agonists and antagonists of lysophosphatidic acid receptors. *Mol. Pharmacol.* **63**, 1032-1042 (2003).
83. Tigyi, G. & Miledi, R. Lysophosphatidates bound to serum albumin activate membrane currents in *Xenopus* oocytes and neurite retraction in PC12 pheochromocytoma cells. *J. Biol. Chem.* **267**, 21360-21367 (1992).
84. Goetzl, E. J. et al. Gelsolin binding and cellular presentation of lysophosphatidic acid. *J. Biol. Chem.* **275**, 14573-14578 (2000).
85. Eichholtz, T., Jalink, K., Fahrenfort, I. & Moolenaar, W. H. The bioactive phospholipid lysophosphatidic acid is released from activated platelets. *Biochem. J.* **291**, 677-680 (1993).
86. Aoki, J. et al. Serum lysophosphatidic acid is produced through diverse phospholipase pathways. *J. Biol. Chem.* **277**, 48737-48744 (2002).
87. Sano, T. et al. Multiple mechanisms linked to platelet activation result in lysophosphatidic acid and sphingosine 1-phosphate generation in blood. *J. Biol. Chem.* **277**, 21197-21206 (2002).
88. Imai, A., Furui, T., Tamaya, T. & Mills, G. B. A gonadotropin-releasing hormone-responsive phosphatase hydrolyses lysophosphatidic acid within the plasma membrane of ovarian cancer cells. *J. Clin. Endocrinol. Metab.* **85**, 3370-3375 (2000).
89. Xu, J. et al. Lipid phosphate phosphatase-1 and Ca²⁺ control lysophosphatidate signaling through EDG-2 receptors. *J. Biol. Chem.* **275**, 27520-27530 (2000).
90. Tokumura, A. et al. Identification of human plasma lysophospholipase D, a lysophosphatidic acid-producing enzyme, as autotaxin, a multifunctional phosphodiesterase. *J. Biol. Chem.* **277**, 39436-39442 (2002a).
91. Umezaki, G. et al. Autotaxin has lysophospholipase D activity leading to tumour cell growth and motility by lysophosphatidic acid production. *J. Cell Biol.* **158**, 227-233 (2002).
92. Sciorra, V. A. & Morris, A. J. Roles for lipid phosphate phosphatases in regulation of cellular signaling. *Biochim. Biophys. Acta* **1582**, 45-51 (2002).
93. Tanyi, J. L. et al. The human lipid phosphate phosphatase-3 decreases the growth, survival, and tumorigenesis of ovarian cancer cells: validation of the lysophosphatidic acid signaling cascade as a target for therapy in ovarian cancer. *Cancer Res.* **63**, 1073-1082 (2003).
94. Xu, Y. et al. Lysophosphatidic acid as a potential biomarker for ovarian and other gynecologic cancers. *JAMA* **280**, 719-723 (1998).
95. Baker, D. L. et al. Plasma lysophosphatidic acid concentration and ovarian cancer. *JAMA* **287**, 3081-3082 (2002).
96. Hama, K., Bandoh, K., Kakehi, Y., Aoki, J. & Arai, H. Lysophosphatidic acid (LPA) receptors are activated differentially by biological fluids: possible role of LPA-binding proteins in activation of LPA receptors. *FEBS Lett.* **523**, 187-192 (2002).
97. Gerrard, J. M. & Robinson, P. Identification of the molecular species of lysophosphatidic acid produced when platelets are stimulated by thrombin. *Biochim. Biophys. Acta* **1001**, 282-285 (1989).
98. Giustina, A., Miceli, D., Dolo, V., Romano, F. M. & Vittorelli, M. L. Membrane vesicles in ovarian cancer fluids: a new potential marker. *Anticancer Res.* **19**, 3439-3445 (1999).
99. Andre, F. et al. Malignant effusions and immunogenic tumour-derived exosomes. *Lancet* **360**, 295-305 (2002).
100. Stracke, M. L. et al. Identification, purification, and partial sequence analysis of autotaxin, a novel motility-stimulating protein. *J. Biol. Chem.* **267**, 2524-2529 (1992).

- Identification of autotaxin as a motogen. Autotaxin would eventually be shown to be lysoPLD, the key enzyme regulating LPA production.**
101. Murata, J. *et al.* cDNA cloning of the human tumour motility-stimulating protein, autotaxin, reveals a homology with phosphodiesterases. *J. Biol. Chem.* **269**, 30479–30484 (1994).
 102. Nam, S. W. *et al.* Autotaxin (NPP-2), a metastasis-enhancing motogen, is an angiogenic factor. *Cancer Res.* **61**, 6938–6944 (2001).
Describes the role of autotaxin in neovascularization in vivo.
 103. Koh, E. *et al.* Site-directed mutations in the tumor-associated cytokine, autotaxin, eliminate nucleotide phosphodiesterase, lysophospholipase D, and motogenic activities. *Cancer Res.* **63**, 2042–2045 (2003).
 104. Clair, T. *et al.* Autotaxin hydrolyzes phospholipids to produce a migration stimulator, lysophosphatidic acid, or a migration inhibitor, sphingosine-1-phosphate. *Cancer Res.* (in the press).
 105. Gijbbers, R., Aoki, J., Arai, H. & Bollen, M. The hydrolysis of lysophospholipids and nucleotides by autotaxin (NPP2) involves a single catalytic site. *FEBS Lett.* **538**, 60–64 (2003).
 106. Bachner, D. *et al.* Bmp-2 downstream targets in mesenchymal development identified by subtractive cloning from recombinant mesenchymal progenitors (C3H10T1/2). *Dev. Dyn.* **213**, 398–411 (1998).
 107. Tice, D. A. *et al.* Synergistic induction of tumour antigens by Wnt-1 signaling and retinoic acid revealed by gene expression profiling. *J. Biol. Chem.* **277**, 14329–14335 (2002).
 108. Croset, M., Brossard, N., Polette, A. & Lagarde, M. Characterization of plasma unsaturated lysophosphatidylcholines in human and rat. *Biochem. J.* **345**, 61–67 (2000).
 109. Nam, S. W. *et al.* Autotaxin (ATX), a potent tumour motogen, augments invasive and metastatic potential of ras-transformed cells. *Oncogene* **19**, 241–247 (2000).
Describes the role of autotaxin in the metastatic cascade in vivo.
 110. Yang, S. Y. *et al.* Expression of autotaxin (NPP-2) is closely linked to invasiveness of breast cancer cells. *Clin. Exp. Metastasis* **19**, 603–608 (2002).
 111. Tanyi, J. L. *et al.* 2003b Role of decreased levels of LPP-1 in accumulation of lysophosphatidic acid (LPA) in ovarian cancer. *Clinical Cancer Res.* (in the press).
 112. Xie, Y., Gibbs, T. C., Mukhin, Y. V. & Meier, K. E. Role for 18:1 lysophosphatidic acid as an autocrine mediator in prostate cancer cells. *J. Biol. Chem.* **277**, 32516–32526 (2002).
Describes an autocrine LPA loop in the stimulation of prostate cancer cells.
 113. Mills, G. B., May, C., McGill, M., Roifman, C. & Mellors, A. A putative new growth factor in ascitic fluid from ovarian cancer patients: identification, characterization and mechanism of action. *Cancer Res.* **48**, 1066–1071 (1988).
 114. Mills, G. B. *et al.* Ascitic fluid from human ovarian cancer patients contains growth factors necessary for intraperitoneal growth of human ovarian cancer cells. *J. Clin. Invest.* **86**, 851–855 (1990).
 115. Xu, Y. *et al.* Characterization of an ovarian cancer activating factor (OCAF) in ascites from ovarian cancer patients. *Clin. Cancer Res.* **1**, 1223–1232 (1995).
Shows that ascites from ovarian cancer patients contain high levels of bioactive LPA, including unusual LPA isoforms.
 116. Westermann, A. M. *et al.* Malignant effusions contain lysophosphatidic acid (LPA)-like activity. *Ann. Oncol.* **9**, 437–442 (1998).
 117. Xiao, Y. J. *et al.* Electrospray ionization mass spectrometry analysis of lysophospholipids in human ascitic fluids: comparison of the lysophospholipid contents in malignant vs nonmalignant ascitic fluids. *Anal. Biochem.* **290**, 302–313 (2001).
 118. Shen, Z., Balinson, J., Morton, R. E., Xu, Y. & Xu, Y. Phorbol 12-myristate 13-acetate stimulates lysophosphatidic acid secretion from ovarian and cervical cancer cells but not from breast or leukemia cells. *Gynecol. Oncol.* **71**, 364–368 (1998).
 119. Okita, M. Abnormal plasma lysophosphatidic acid level in ovarian cancer patients. *Bulletin of Faculty of Health and Welfare Science, Okayama Prefectural University* **1**, 29–35 (1994).
 120. Sasagawa, T., Suzuki, K., Shiota, T., Kondo, T. & Okita, M. The significance of plasma lysophospholipids in patients with renal failure on hemodialysis. *J. Nutr. Sci. Vitaminol.* **44**, 809–818 (1998).
 121. Sasagawa, T., Okita, M., Murakami, J., Kato, T. & Watanabe, A. Abnormal serum lysophospholipids in multiple myeloma patients. *Lipids* **34**, 17–21 (1999).
 122. Okita, M., Gaudette, D. C., Mills, G. B. & Holub, B. J. Elevated levels and altered fatty acid composition of plasma lysophosphatidylcholine (lysoPC) in ovarian cancer patients. *Int. J. Cancer* **71**, 31–34 (1997).
 123. Yoon, H. R., Kim, H. & Cho, S. H. Quantitative analysis of acyl-lysophosphatidic acid in plasma using negative ionization tandem mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **788**, 85–92 (2003).
 124. Shen, Z. *et al.* Fatty acid composition of lysophosphatidic acid and lysophosphatidylcholine in plasma from patients with ovarian cancer and other gynecological diseases. *Gynecol. Oncol.* **83**, 25–30 (2001).
 125. Tokumura, A. *et al.* Lack of significant differences in the corrected activity of lysophospholipase D, producer of phospholipid mediator lysophosphatidic acid, in incubated serum from women with and without ovarian tumors. *Cancer* **94**, 141–151 (2002).
 126. Deng, W. *et al.* Lysophosphatidic acid protects and rescues intestinal epithelial cells from radiation- and chemotherapy-induced apoptosis. *Gastroenterology* **123**, 206–216 (2002).
Indicates that compounds that mimic LPA could decrease toxicity associated with cancer therapy under appropriate conditions.

Acknowledgements

We would like to thank E. Goetzl (University of California, San Francisco) as well as members of our laboratories for allowing us to share unpublished data. This work is supported by grants from the National Cancer Institute (USA), Department of Defense (USA) and the Netherlands Cancer Institute (Holland).

Online links

DATABASES

The following terms in this article are linked online to:
 Cancer.gov: <http://www.cancer.gov/>
 breast cancer | cervical cancer | endometrial cancer | head and neck | melanoma | myeloma | ovarian cancer | prostate cancer
 LocusLink: <http://www.ncbi.nlm.nih.gov/LocusLink/>
 ATX | LPA1 | LPA2 | LPA3 | LPA4 | PLA1 | PLA2 | PLC | PPARγ | RAS | TIAM1